

XII. Laboratory Services

A. State Public Health Laboratory / Mycobacteriology

1. Submitting specimens:
 - a. Use DHHS 1247 <https://slph.ncpublichealth.com/Forms/1247-Mycobacteriology-TB-20170801.pdf> to order supplies from:
Laboratory Mailroom, Division of Laboratory Services
P. O. Box 28047
Raleigh, NC 27611-8047
Courier # 52-41-41
Phone: 919-733-7656
 - b. Provide all requested information, including Medicaid number and social security number.
 - c. Consult the State Laboratory Manual **SCOPE** for detailed information.
2. Laboratory results:
 - a. First-time smear positive results are reported by telephone to the provider who submitted the specimen on the day the specimen is processed.
 - b. Smear results are reported as:
 - No acid-fast bacilli found or,
 - Acid-fast bacilli found (<1 **or** 1-10 **or** >10 per field).
 - c. Preliminary culture results are reported as:
 - Growth suggestive of *Mycobacterium tuberculosis*.
 - Growth suggestive of nontuberculous mycobacterium (NTM).
 - d. Final culture results are reported as:
 - "Growth identified as *Mycobacterium tuberculosis* complex"
 - i. (*M. tuberculosis*, *M. bovis*, *M. africanum*).
 - "Growth identified as Mycobacterium (species name)."
 - "Contaminated", indicating overgrowth with bacteria or other microorganisms.
 - e. No further action is required for a nontuberculous mycobacterium (NTM) results UNLESS the patient is currently under treatment as a TB suspect.
3. Susceptibility testing:
 - a. Performed on all initial positive *M. tuberculosis* cultures for:
 - Isoniazid
 - Rifampin
 - Pyrazinamide
 - Ethambutol
 - b. Performed for second line drugs if resistance to any first-line drug is present.
 - c. Reported as "S" for Susceptible or "R" for Resistant for liquid media (e.g. Bactec).
 - d. Reported as a percent (%) resistance (colony count) for solid media results.

- e. Performed for second-line drugs (streptomycin, rifabutin, ciprofloxacin, kanamycin, ethionamide, capreomycin, PAS, ofloxacin, amikacin) by special request.
4. Notification to the health department:
 - a. Original report sent to the health department on specimens submitted by the health department.
 - b. "County copy" sent to the health department for smear positive and/or *M. tuberculosis* culture specimens that were submitted by other health care providers.
 - c. Reported by Electronic Laboratory Report (ELR) in NCEDSS.
5. *M. tuberculosis* cultures are retained by the laboratory for one year.
6. North Carolina is participating in a universal genotyping program through the State Public Health Lab. Therefore, private and hospital-based laboratories that process their own cultures need to forward one specimen per *M. tuberculosis* culture positive patient to the NC State Public Health Lab. Local TB nurses need to help ensure that this process takes place.
7. Private Laboratory Mycobacteriology
 - a. All laboratories are required by G. S. 130A-139 to report positive smears and *M. tuberculosis* cultures within seven days of obtaining the result.
 - b. Reports from private and commercial laboratories (Report of Positive Smear (AFB) and/or Positive Culture of *M. tuberculosis* - DHHS 3005) https://epi.publichealth.nc.gov/cd/tb/docs/dhhs_3005_2017.pdf are sent to N.C. TB Control .
 - c. N.C. TB Control forwards these reports to the patient's county of residence.
 - d. Contact the local nurse consultant with questions upon receipt of results from a private laboratory.
 - e. As noted above, one specimen per *M. tuberculosis* culture-positive patient should be forwarded to the State Public Health Lab for genotyping.
8. Nucleic Acid Amplification Testing (NAA) For Tuberculosis: North Carolina State Laboratory of Public Health Testing and General Guidelines for Interpretation:
 - a. In some instances, NAA test results can provide a more rapid diagnosis of tuberculosis (or exclude such a diagnosis), allowing practitioners to make more informed decisions regarding treatment and the need for isolation. NAA tests are designed to supplement, rather than to replace, standard mycobacterial culture for confirmation of diagnosis and the test is not suitable for all specimens.
 - b. Types of NAA tests available:
 - MTD (Gen-Probe®) is FDA approved for detecting *M. tuberculosis* in smear-positive AND smear-negative respiratory specimens from patients suspected of having tuberculosis.

- Amplicor® is another type of NAA test but it is **only** FDA approved for detecting AFB smear-positive respiratory specimens; potential advantages of NAA tests include more rapid diagnosis, but, as noted above, they do NOT replace AFB smear or mycobacterial culture, and do not replace clinical judgment.
 - GeneXpert TB® is FDA-approved for detecting *M. tuberculosis* in smear-positive and smear-negative respiratory specimens in patients suspected of having tuberculosis who have had 3 or fewer days of antituberculous treatment. This assay is the assay used by the N.C. SLPH as of early 2020.
- c. The N.C. SLPH will perform an NAA test on undigested primary clinical specimens only (i.e., directly on the sample, not on samples already set up for culture). The sample will undergo routine processing to determine if it is AFB smear-positive or negative.
- d. NAA testing will be performed on all first-time AFB smear-positive specimens for each patient (whether requested or not).
- e. NAA testing will only be performed on smear negative specimens in exceptional circumstances.
- f. When submitting respiratory specimens to the SLPH submit three spontaneous or induced sputum specimens initially (early morning samples are best), or a sample from BAL in addition to a pre- and post-BAL specimen. Specimens should be sent to the SLPH as soon as possible after a specimen is collected. Refrigerate specimens after obtaining them.
- g. Extra-pulmonary specimens:
- Selected specimens from extra-pulmonary sites can be submitted for NAA testing. Specimens appropriate for testing include lymph node aspirate or biopsy, tissue biopsy, and urine. NAA testing has a high specificity on smear-positive specimens and can confirm a diagnosis of active TB in such instances. However, the sensitivity is much lower, especially on smear-negative specimens, so the **NAA test result cannot be used to exclude a diagnosis of active TB if the specimen is AFB smear-negative**. Therefore, unless you have specifically discussed with the SLPH and gotten approval, only smear-positive tissue specimens will be tested using an NAA test.
 - Specifically, pleural fluid and cerebrospinal fluid (CSF) are generally not appropriate specimens for NAA testing, as these specimens are generally smear-negative and NAA tests have very low sensitivity. Smear-positive pleural fluid or CSF can be submitted for testing.
- h. Interpretation of NAA tests

- NAA tests can be useful to rapidly determine whether a patient poses an infectious risk to others, particularly in instances where a patient would otherwise be isolated waiting for culture confirmation of a diagnosis (e.g. in a hospital setting). A negative NAA test on these samples does not completely exclude the presence of TB; however, a negative test does indicate that the quantity of *M. tuberculosis* in the sputum is a very low level, so the patient is less likely to be contagious. It should not be used in place of sound clinical judgment when determining matters of infectivity or the need for isolation.
- NAA tests are **not licensed** for use on non-respiratory tissues or specimens (e.g., cerebrospinal fluid, lymph node tissue), should be interpreted with extreme caution if ordered for one of these non-licensed uses, and should not be used to “rule-out” TB in these circumstances.
- Interpretation of an NAA test done on an approved specimen:
 - In general, a “positive” NAA test should be considered evidence of *M. tuberculosis* disease, while awaiting final culture results;
 - A “negative” NAA test from a smear-positive sputum specimen suggests nontuberculous mycobacterial infection. Performing a second NAA test on a second smear-positive specimen is recommended in this case
 - A “negative” NAA test from a smear-negative sputum specimen is not sensitive enough to rule out TB disease; clinical judgment should be used for further evaluation and treatment decisions

For more information about NAA testing, contact your regional TB Nurse Consultant, or refer to the following CDC publications related to information on NAA testing for tuberculosis:

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm?s_cid=mm5801a3_e

https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6241a1.htm?s_cid=mm6241a1_e

<https://www.cdc.gov/MMWr/preview/mmwrhtml/mm6407a8.htm>

https://www.cdc.gov/tb/publications/factsheets/testing/xpert_mtb-rif.htm

9. Sputum Collection Procedure

- a. Collect three sputum specimens initially; preferably early in the morning. At least one should be observed if possible. Specimens should then be collected every two weeks until culture conversion to negative. Occasionally, it may be necessary to collect sputum weekly when trying to discontinue isolation.
- b. Instruct the patient/family as follows:
 - Thoroughly rinse mouth with water before collecting specimen
 - Collect only material coughed up from the lungs – not saliva or post-nasal drainage.
 - Collect specimen(s) upon arising on three successive days.
 - Inhaling steam, e.g. hot shower, may help induce sputum production.
 - Collect at least 5 ml of sputum (1 teaspoon).
 - Expectorate directly into the plastic tube and thoroughly dry the lip of the tube before screwing the cap on tightly.
 - Refrigerate the specimen until specimen is given to the health department.
- c. Label plastic specimen tube with the patient's name and identification number that matches the requisition form DHHS 1247 (<https://slph.ncpublichealth.com/Forms/1247-Mycobacteriology-TB-20170801.pdf>).
- d. The completed DHHS 1247 should be placed around the outside of the inner metal container.

10. Sputum Induction Protocol

- a. The purpose of sputum induction is to obtain a sputum specimen for diagnostic or sputum conversion purposes from a patient unable to produce a specimen naturally.
- b. This procedure can be done outside patient's home or within the home in a well-ventilated area.
- c. If procedure is performed in a healthcare facility, a room, booth or enclosed area must have negative pressure. Airflow must be from the corridor into the sputum induction room then exhausted to the outside.
- d. Equipment required:
 - Nebulizer and disposable "neb kit" (tubing, mouthpiece, plastic chamber for sterile hypertonic saline);
 - Sputum container properly labeled;
 - Sterile, non-bacteriostatic (no preservatives) 3%-10% hypertonic saline (5-10cc); and
 - N95 respirator or equivalent.

- e. Preparation of equipment:
- Inspect nebulizer for cleanliness. If necessary, wipe nebulizer surfaces with a 10 percent bleach solution.
 - Place hypertonic saline in the nebulizer chamber.
 - Connect mouthpiece to tubing (including chamber) and connect tubing to machine.
 - Test nebulizer by turning equipment on and observing mist production.
- f. Preparation of patient:
- Explain procedure to patient and demonstrate nebulizer function
 - Have patient rinse mouth out with a disposable cup of water.
 - Instruct patient to place his/her lips around the mouthpiece and inhale the aerosol, through the mouthpiece, using slow, deep breaths for 10-15 minutes.
 - Instruct patient to cough vigorously if spontaneous coughing does not occur; the patient should cough several times and expectorate all sputum into the container.
 - Instruct patient to keep sputum container closed until ready to expectorate; direct patient to close container securely after the specimen has been collected.
 - Caution patient not to leave the sputum induction area until coughing has completely stopped.
 - Instruct patient to shut the door after leaving the sputum induction room.
- g. Staff role
- Staff must wear properly fitted respirator while in the sputum induction room.
 - Verify patient is using nebulizer correctly.
 - It is not necessary for staff to remain with patient during procedure.
 - Staff must remain nearby to assist patient if necessary and to ensure patient remains in the sputum induction room until coughing has stopped.
- h. Preparation of specimen:
- Sputum may appear watery. Send to lab regardless of appearance.
 - Properly label plastic container with patient's name and identification number that matches the requisition form DHHS 1247.
 - Check "induced sputum" on the lab requisition.
 - Prepare two sputum containers for patient to take home, making sure they are properly labeled.
 - The induction procedure may cause patient to produce sputum later. If patient produces a specimen later in the

day (following induction), the specimen should be sent to the lab.

- i. Care of equipment and area:
 - To determine length of time required to decontaminate air, refer to Centers for Disease Control and Prevention. Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health Care Facilities, MMWR 1994/ 43(RR13), page 72.
 - If staff enters room prior to the end of the time period determined in “a” above, a properly fitted respirator must be worn.
 - Never reuse disposable nebulizer tubing or mouthpiece. Seal used articles in a plastic bag and discard in biohazardous receptacle.

11. Gastric Aspiration Procedure

Gastric aspiration is the recommended method of collection of respiratory secretions for the bacteriologic diagnosis of TB disease in children who are unable to expectorate sputum. Compared with bronchoalveolar lavage (BAL), gastric aspiration is less invasive, has fewer potential complications, and is an excellent method for confirming a bacteriologic diagnosis. The specimens should be collected as follows:

- a. Patients should be hospitalized whenever possible to ensure proper collection and processing of gastric aspirates. If done on an outpatient basis it should be done before the patient takes anything by mouth, sits up or ambulates.
- b. The microbiology laboratory should be notified of the anticipated collection of specimens and a technician should be identified to facilitate processing of the specimens.
- c. Early morning specimens should be collected on three consecutive mornings.
- d. The patient should fast 6 to 8 hours before the procedure. He/she should have no intake of food (solids, liquids, etc.) from 10 p.m. until after the specimen is collected at 6 a.m. the following morning.
- e. A feeding tube (8 FR) should be placed into the stomach while the patient is recumbent, preferably while still asleep or immediately upon awakening. Once the location of the tube is verified a minimum of 5 ml of gastric contents should be aspirated with a 10-20 ml syringe.
- f. A minimum of 5 ml of gastric contents is needed for processing. If less than 5 ml are aspirated, 20-30 ml of non-bacteriostatic sterile water (not tap water) should be inserted through the tube and the

gastric contents should be aspirated. The feeding tube should then be removed.

- g. Specimens should be placed in a sterile container and **immediately** adjusted to a neutral pH with 100 mg of sodium carbonate (Na_2CO_3) salt or with 3 ml of a sodium bicarbonate solution (100 mg/ml). The neutralization step may be completed at the bedside or the specimen may be transported **immediately** to the laboratory and neutralized on arrival.
- h. All specimens should be transported to the microbiology laboratory **immediately** or, if neutralized at the bedside, should be refrigerated until transport to the laboratory.

12. Serum Concentration Levels for TB Drugs

Serum drug levels (SDL) should be considered for the following patients:

- HIV-infected patients with a CD4 count of < 100 cells/mm³ who are taking isoniazid and/or rifampin as part of the TB regimen.
- Apparent treatment failure (recurrent positive culture during therapy).
- Patients who have completed a full course of TB treatment and who experience TB relapse within two years
- Persistently positive AFB smear or culture after 12 weeks of DOT (85 percent of patients should have negative cultures within eight weeks).
- Known gastrointestinal conditions, surgical procedures or abnormalities likely to interfere with medication absorption, e.g., partial gastrectomy, Crohn's disease, resection of small intestine.

Send specimens to the Infectious Disease Pharmacokinetics Laboratory at the University of Florida. The cost for each assay historically has been about \$80. This can be billed to the health department or the health department can send a check with the specimen. To have it billed to the health department, write in the health department's name, address, phone and fax numbers in the responsible party section and they will bill you. Call your TB Nurse Consultant if you want to access these funds. Complete one column of information for each drug to be assayed. Make as many photocopies as you need. All specimens sent to the laboratory should conform to all Federal and IATA shipping regulations. If you have any questions call the Infectious Disease Pharmacokinetics Laboratory at (352) 273-6710. The requisition form and instructions can be found at the following link <https://idpl.pharmacy.ufl.edu/forms-and-catalog/>

- 13. The NC State Laboratory Mycobacteriology requisition form (DHHS 1247) can be found at: <https://slph.ncpublichealth.com/Forms/1247-Mycobacteriology-TB-20170801.pdf>
- 14. The Report of Positive (AFB) and/or Positive Culture of M. Tuberculosis (DHHS 3005) can be found at: https://epi.publichealth.nc.gov/cd/tb/docs/dhhs_3005.pdf